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XXIV.—*On the Presence of Chlorophyll-cells and Starch-granules as Normal Parts of the Organism, and on the Reproductive Process, in* Difflugia pyriformis, *Perty; also on a Freshwater Species of* Echinocystidia. By H. J. Carter, F.R.S. &c.

For some time past I have been inclining to the view that, although the Rhizopoda must be placed on the animal side of organized beings, still they have very strong alliances with the vegetable kingdom; and this opinion has been confirmed by what I have lately observed in *Difflugia pyriformis*, Perty (mihi), wherein the body, apparently after conjugation similar to that of the contents of the cells of *Spirogyra*, contracts itself into an elliptical form, densely charged with chlorophyll-cells and starch-granules. I cannot now state confidently that the contents of both tests, after amalgamation, finally become fixed in one only, as in *Spirogyra*; nor, on the contrary, am I prepared to state that they become divided between the two individuals, for reasons which will be mentioned hereafter; but this much may be stated—that the number of chlorophyll-cells and starch-granules greatly increases in the body of this *Difflugia* just previously to subsequent changes, in which they seem to disappear altogether (at all events, the former) and are replaced by a mass of colourless granuliferous cells which may be inferred to be the elements of a new generation.

Before, however, proceeding to that which I have to offer on the subject, it is desirable to describe the species which is about to come under our consideration; and this may stand as follows:—

Difflugia pyriformis, Perty.

Test ovate-elongate, closed and rounded posteriorly, open and truncate, with even or undulating aperture, anteriorly; composed of hyaline grains of quartz-sand, held together by a glutinous (albuminous?) substance. Animal consisting of diaphane and sarcode, the former disposed in a transparent layer around the latter, and sending off processes of attachment from its posterior third to the inner surface of the test; sarcode more or less clouded by the presence of chlorophyll-cells, which impart a green colour to the body, by the "moleculæ," the "granules," and portions of food in process of digestion. Granules abundant, minute, colourless. Nucleus situated in the posterior end of the body, fixed, and consisting of a transparent spherical cell bearing on one part of its inner surface the nucleolus in the form of a circular, colourless, opake, discoid body much less in diameter than the nuclear cell. Contracting vesicles or "vesiculæ" not seen, but probably in plurality, and situated round the border of the posterior end, as in *D. tricuspis*, Cart.

Hab. Fresh water, in stagnant pools, with decaying leaves and vegetable matter. Active in the spring (April), passive and more or less retracted within the test in the autumn (August). Locomotion and capture of food performed by digital prolongations of the body slowly projected through the aperture of the test, and into which the chlorophyll-cells do not enter.

Size. Length $\frac{1}{60}$th, greatest breadth $\frac{1}{150}$th, and width of aperture $\frac{1}{360}$th of an inch. Thus the test is a little longer than twice its breadth. In upwards of 200 specimens the measurements varied very little from those given.

Loc. England, south coast of Devonshire.

Observations.—I learn from MM. Claparède and Lachmann (Etudes sur les Infusoires et Rhizopodes, p. 448, for a copy of which work, received since my return from India, I now beg to acknowledge myself under great obligation to the authors—the latter, alas! removed by death too soon for the interests of science) that there is a *Difflugia pyriformis*, Perty (Zur Kenntniss, &c., p. 187, pl. 9. ob. Abth. f. 9); and I see a figure of *D. pyriformis* in pl. 21. f. 17 of Pritchard's 'History of the Infusoria' (ed. 1861) without further mention, but so much like the one which I have above described, that, on the evidence altogether, I do not hesitate to call the Devonshire specimens *D. pyriformis*, Perty. Also, among upwards of 200 specimens of *D. pyriformis*, I have only found five with that diverticulum at the posterior end which led Ehrenberg to call it *D. acuminata*, but in other respects so like, in the *green* body and sandy composition of the test, that I cannot help thinking it is only a variety of *D. pyriformis*. Each of these five, however, were only about $\frac{1}{100}$th of an inch long, including the diverticulum, while *D. acuminata* (ap. Pritchard) is set down at $\frac{1}{70}$th of an inch in length. Lastly, among the 200 were also three or four subglobose specimens, like *D. proteiformis*, Ehr., which are set down as $\frac{1}{240}$th of an inch in Pritchard (p. 553), but mine averaged only $\frac{1}{900}$th of an inch in length; yet in April last I found two others in the same place larger than Pritchard's measurement, viz. $\frac{1}{160}$th of an inch long; so that all three of these *Difflugiæ* are found together here; and although the latter, in the few specimens of it which I obtained, did not present the green colour of the two former, yet it is so like them in every other respect, that I cannot help thinking that *D. acuminata* and *D. proteiformis* are but small varieties of *D. pyriformis*, which is by far the largest in body of all, because the measurement assigned to *D. acuminata* (viz. $\frac{1}{70}$th of an inch) probably includes the diverticulum. Still the Rhizopoda vary so much both in size and form, that the tailed variety may be the largest, and the pyriform one subordinate in size and number, or the subglobose one, and so on, in another locality. In the present instance, however, it is *D. acuminata* and *D. proteiformis* which are so very subordinate in size and number. The absence of the green colour in *D. proteiformis* may have taken place in the process of generation, as it will hereafter be shown to do in *D. pyriformis*; at the same time, if future evidence should prove that it is never green, then it will be necessary to regard *D. proteiformis* as a different species; for the green colour in *D. pyriformis* and *D. acuminata* is due to the presence of chlorophyll-cells, as much as the green colour in the body of *Hydra viridis*.

Of the 200 specimens of *D. pyriformis* mentioned, there were eighteen colourless ones, owing, as just stated, to the total absence of chlorophyll-cells, which appears to indicate the stage in the generative process to which I have just alluded.

There were also fourteen empty tests (and I have found many more since, with a less number of filled ones), which may have arisen from their contents having left them during the process of conjugation, or, in a more advanced stage of the generative process, from the old animal having become effete, and the new generation having left the test, or from the death of the animal accidentally. The number, however, is so much out of proportion to the filled tests, both green and colourless, that it stands much against the possibility of the conjugation being exactly like that of *Spirogyra*, *i. e.* of the result of this conjugation being always to leave one cell empty.

All my specimens were collected from the same place where I found *Amœba princeps* in April last (see Annals, vol. xii. p. 30, 1863), and in the following way, viz. by taking up the surface of the bottom of the little pools of water among the dead leaves with an india-rubber bottle and tube, and transferring it to a glass bottle, then taking out the sediment by portions with a large hair pencil, and spreading it over a glass slide with a piece of white paper under it for the green, and one of black for the colourless specimens, after which the *Difflugiæ* may be easily recognized with a magnifying-glass, separated with a needle, and finally transferred to some clean water previous to further examination.

On the sides of the bottle holding the sediment I have observed several specimens at different heights, and all green but one, which was colourless; besides, all the specimens which I have crushed under the microscope have contained portions of food; from which circumstances both green and colourless specimens may be assumed to be still more or less active, although not near so much so as in the spring of the year,—thus not differing from other beings (as will be seen hereafter) in continuing to take in nourishment throughout the greater part of the generative process. During the time that the body is densely charged with chlorophyll-cells, and the grains of sand of which the test is composed are thick and irregular, it is impossible to see the different parts of the animal *in situ*; hence it was only a chance specimen which fell under my eye in April last, from the locality to which I have alluded, that, with few grains of sand on it, and very few chlorophyll-cells in the interior, then permitted me to make the drawing from which the above description has been taken.

It is remarkable, too, that in no instance have I yet found a *coloured* grain of sand in the test; all have been composed of hyaline quartz, as if the animal had exerted a choice in this respect—a choice of those particles only which allowed the light to pass through them uninterruptedly.

Further, with the exception of *one* adult specimen of *Amœba princeps*, I have not seen a single *Amœba*, large or small, during these examinations, where, in April last, *A. princeps* so abounded. It should perhaps be added that there had been very little rain previously for many days, the water had become low, and there was no development, at this place, of the Confervoid Algæ, which, on decomposing, afford so much nutriment to the Rhizopoda. Whether this, or the season of the year, has led to the scarcity of *Amœbæ* at this time or place I have not yet had an opportunity of proving.

With this short introduction, let us turn our attention, first, to the composition of the green spore-like body, as it is now found in the tests of *Difflugia pyriformis*, and then to the colourless one; for which purpose it will be necessary to remove one of the *Difflugiæ* with the green body (as that is the first to be examined) to a slide, with a little water, and then cover it with a light thin bit of glass; after which it should be placed under the microscope, and, watching it while it gradually becomes crushed and its contents issue, by abstracting part of the water with a little bibulous paper we shall observe that these contents are composed of—

1. A small quantity of thin protoplasm, with its imbedded "moleculæ," which suspends and holds together the general mass. This frequently oozes out, too, in spherical portions, of different sizes, each of which may contain more or less of the moleculæ, but must not be mistaken for separate cells—the variety in size helping to show that they do not belong to any special set of cell-organs.

2. A great number of spherical cells, of a fresh green colour, about $\frac{1}{6000}$th of an inch in diameter, containing chlorophyll and granular protoplasm.

Iodine with sulphuric acid causes their contents to assume a dark brown colour. Sulphuric acid alone gives them first a sea-green or bluish-green tint, and then extracts the colour*.

These cells are exactly like the chlorophyll-cells of *Hydra viridis*, with the exception of being a little smaller. Fortunately this animal was present for me to make the comparison.

3. A nearly equal number of colourless refractive granules, of globular, oval, and irregularly round forms, more or less compressed, and of different sizes, varying from $\frac{1}{3000}$th to $\frac{1}{600}$th of an inch in their greatest diameter respectively, the smallest being the most numerous.

Very diluted sulphuric acid, followed by iodine, gives them for the most part a deep claret colour, and, to many, very frequently the deep blue colour characteristic of genuine starch. Strong sulphuric acid, preceded by iodine, causes on its approach much blue colour to appear, indicative of the presence of amorphous starch in the mass, which colour disappears on the strong acid reaching it; it also causes a pellicle to appear on the large granules, which swell up and, bursting, frequently display a crevice in the centre, or radiating cracks which show that the interior of the granule is filled with a homogeneous, semitransparent, pulpy substance; while the small granules lose all their colour, but the pellicle remains, and still retains their forms respectively. Indeed the indications, both physical and chemical, in these granules, of an amylaceous composition are so strong, that although the genuine blue colour may not always be brought out in them by chemical means, yet no doubt can remain that they are starch in some form or other.

4. There are also a few oil-globules frequently present; but

* By "iodine" I mean a solution of iodine in one of iodide of potassium.

the great bulk of the mass is composed of the chlorophyll-cells and starch-granules in nearly equal proportions.

5. The nucleus is no longer a discoid semiopake body attached to the inner surface of a transparent spherical cell; but this spherical cell, which is now about the $\frac{1}{461}$st part of an inch in diameter, is filled (lined?) with refractive spherules, about $\frac{1}{6000}$th of an inch in diameter, mingled with minute granules and protoplasm. These contents are *now* adherent to the external thick cell of the nucleus (or "nuclear utricle," as it has been termed by Nägeli), but subsequently become separated from it, while in one part of the spheruliferous mass may be observed a small transparent area, about $\frac{1}{1500}$th of an inch in diameter, which appears to be the nucleolus: the last is compressed in shape, and will hereafter be found to form a more intimate bond of union between the spheruliferous mass and nuclear utricle than any other part. The spheruliferous mass seems to be a thus altered state of the transparent protoplasm of the nucleus, and not an increased development of the opake nucleus, as I formerly thought.

Here I would take the opportunity of correcting what now appears to be an error in my description of the nucleus in *Amœba princeps*, viz. that whereas I have viewed the "transparent area" there as caused by the *nucleolus* partially spreading over the inner surface of the nucleus or nuclear utricle, from the opposite point, and thus leaving this area, I would now regard the "transparent area" as I have done that one which is similar to it in the granulated nucleus of *Difflugia pyriformis*, viz. as the nucleolus, and the opake portion as a thus altered state of the transparent protoplasm of the nucleus, which ultimately becomes granuliferous both in the "reproductive cells" and in what I have termed the "granulation of the nucleus." This, too, will, I think, accord better with Dr. Wallich's figure of the nucleus in his *Amœba villosa* (Annals, vol. xi. pl. 9. fig. 7, 1863); for I, of course, do not regard this condition now as the primitive state and form of the nucleus, which is that probably of a discoid opake body attached to the inner surface of a transparent globular vesicle, as in other cells, but, on the contrary, as the first phase of its generative development. Hence my assumption that the latter state may form a specific character for *A. princeps* falls to the ground, and the villous tail, first pointed out by Dr. Wallich, may prove a better indication.

Iodine causes the nucleus of *Difflugia pyriformis*, in the state last described, to assume a light amber-colour, which passes into a violet tint with undiluted sulphuric acid, when the whole body suddenly swells up, but does not burst, the spherular structure is destroyed, and the violet tint appears to be deepest in the situation of the nucleolus. Iodine alone, however, does not alter the colour of the spherules, even when, by forcibly bursting the nucleus, they are pressed out into the surrounding liquid.

6. Lastly, portions of food are always present, which show, by the yellow and brown state of their chlorophyll, that they are undergoing digestion, while they thus contrast strongly with the fresh green colour of the chlorophyll-cells, which form part of the organized structure of the *Difflugia*.

Thus we have gone through the contents of the body of *Difflugia pyriformis* under its *green* colour, and have seen that the bulk of these contents chiefly consists of chlorophyll-cells and starch-granules. Let us now examine the body in the colourless animal, where we shall find our attention drawn from the striking characters just mentioned, which so strongly connect *D. pyriformis* with the vegetable kingdom, to that part in the economy of this species which is intimately connected with the process of generation.

Taking, then, one of the colourless specimens and placing it in the field of the microscope under similar circumstances to the green one, we shall observe that in the body there is now—

1. The same kind of protoplasm as in the green state, but much more plentiful and much more plastic.

2. An *entire* absence of the green or chlorophyll-cells.

3. The number of starch-granules more or less reduced.

4. Little or no appearance of oil-globules.

5. The greater part of the bulk of the mass, now consisting of small, colourless, acapsular, granuliferous cells, of a globular or oval shape, about $\frac{1}{6000}$th of an inch in diameter, most of which are undergoing multiplication by duplicative division, in which condition they are just twice the length of the single ones. These bodies are smaller in some colourless specimens than in others, indicative of an earlier state of development.

Iodine gives them a light amber tint, which is slightly deepened by sulphuric acid.

6. The nucleus, of the same size as in the green specimens, viz. the $\frac{1}{461}$st part of an inch in diameter, but now more or less *effete*, inasmuch as the spherules have nearly or wholly disappeared, as the case may be, from the nuclear protoplasm, and have left a delicate deciduous structure, apparently sacciform, which, by the aid of chemical tests, is now found to be detached from the nuclear utricle at all parts, except where the nucleolus still connects it with this utricle. But the nucleolus, now that it can be more distinctly viewed, is seen to be composed of a circular compressed cell, which, while it unites the effete protoplasm to the nucleus, also presents a number of small spherules

in *its* interior, within which, again, is another cell or transparent area: that is, the nucleolus appears to be composed of its proper cell, then a protoplasm in which *its* spherules are developed, and then within this again a central cavity filled with some transparent fluid—being now, in fact, only a repetition in structure of the nuclear utricle which surrounds it, and of the vegetable cell generally.

Out of the fourteen colourless specimens, only two afforded me the opportunity of seeing the effete nucleus, it having previously disappeared in the rest through atrophy, or having become destroyed by contact during compression with the rough grains of sand of which the test is composed—a frequent occurrence in examining the contents of the green specimens.

One of these effete nuclei represented the description just given; and the other differed from it only in a large portion of the grumous contents of the nuclear protoplasm still remaining in it, but no spherules, while the nuclear utricle had become ruptured at one point, and a small portion of the nuclear protoplasm, now presenting a sacciform appearance, entangled in it, to which one of the granuliferous cells above mentioned adhered outside, and so strongly that it could not be separated by any means that would not destroy the whole structure of the nucleus. This granuliferous cell I therefore infer to have been one of the spherules, which was still so far united to the parent protoplasm; but whether the rupture was caused by the pressure to which the nucleus had been subjected, or had occurred naturally, to allow of the passage of the spherules from the nucleus into the body of the animal (as appears to be the case in the process of reproduction in the Rhizopodous cell which inhabits the protoplasm of *Nitella*, of which more hereafter), remains to be determined. There was also an appearance, on the external surface of the nucleus, of a minute hole extending through the nuclear utricle to the centre of the nucleolus; but whether, again, this was caused by the presence of the transparent area in the centre of the spherules of the nucleolus, or by a real hole, I was not able to decide. In much of this examination I was greatly assisted by the use of iodine and sulphuric acid, which, it should be remembered, must be used cautiously, and time given to them to produce their respective effects, or they will fail to elucidate that amount of structure which otherwise may be brought out by their application.

7. Lastly, surrounding the whole animal portion in one specimen of the colourless *Difflugia*, was a tough transparent membrane like a capsule, which, tested with iodine and strong sulphuric acid, showed a violet tint, and seemed to have been secreted for the protection of the young; but, as it only occurred in one

instance, I merely mention the circumstance for what it may hereafter prove worth.

Thus, from the granuliferous cells having been *added* to the animal mass, and their being smaller in some specimens than in others, owing apparently to an earlier stage of development, the absence of the spherules in the nucleus while it presented an effete form, and the diminution in the quantity of starch-granules—to say nothing of the total disappearance of the green or chlorophyll-cells, and the presence, in one instance, of a capsular membrane—the colourless specimens of this *Difflugia*, altogether, afford strong evidence of that stage of generative development in which the young brood, as yet without the power of locomotion, have passed from the nucleus into the body of the parent for further development.

Since the above was written, I have examined eight more colourless specimens with the same results. In four of these only, however, was the nucleus seen, and in all it was more or less emptied of the spherules, but not sufficiently well situated for more extended examination with chemical tests.

Of the value of the conjugation in this process it is not in my power to state more than that two tests become united at their apertures, and that in the pair which I found thus united the chlorophyll-cells were still present, and the whole of these, that is, the green matter at least, had gone over to one individual; but subsequently, part returned to their original test, and a flow of contents backwards and forwards between the two tests, which could be plainly witnessed under the microscope, went on for a whole day after they were first discovered in conjugation; at the end of which the tests separated, and the green or chlorophyll-cells became so unequally divided, that one test only contained one-fourth and the other the remaining three-fourths of these organs,—with what amount of the rest of the contents could not be seen.

This is similar to what I have already figured and described in *Euglypha* (Annals, vol. xviii. p. 230, 1856), observing that, "When we find *Euglypha* as well as *Arcella* united not only in pairs, but triply and quadruply in this way, and the same with *Euglena viridis*, the connexion of these phenomena with reproduction, as Claparède has stated, becomes exceedingly doubtful." Now the same kind of plurality in conjugation may, and probably does, take place with *Difflugia pyriformis*; but, then, has it no analogy with other organisms, where reproduction is evidently the effect of conjugation? Certainly in *Spirogyra*, wherein the protoplasm becomes richly charged with chlorophyll and starch just previous to conjugation, we occasionally see two cells tubulating towards one, and one towards two, or the whole of the contents of one cell going over to mingle with those of another, to form the rounded or elliptical spore, as the case may be; or a portion only of the contents doing this, that is, the mass ultimately becoming unequally divided, while *each* portion assumes the rounded spore-form in its respective cell; or an arrest of the process after union of these contents, when the two portions remain connected by an isthmus band through the tube of intercommunication; or, finally, the contents of each cell assuming the spore-like form in their respective cells, without ever mingling at all, &c. Nay, I have drawings of a *Spirogyra* trying to tubulate with the cells of a filament of *Cladophora*,—that is, the tube of intercommunication of the cells of the filament of the *Spirogyra* respectively being projected against those of the *Cladophora*, but ending upon the latter in a bunch of cæcal tubuli which present a rootlike appearance in each instance.

I have not data to state, as before mentioned, whether, in the conjugation of *Difflugia pyriformis*, the whole of the contents of the two individuals *normally* remains in one test or not; for, in the instance mentioned, although this was the case at first, the contents, as I have stated, subsequently became very unequally divided; nor have I found a sufficient number of the empty tests among the filled ones to indicate this; but as regards the union of more than two tests together throwing doubt upon the view that such is a true act of generative conjugation, we have seen that there is just as much variety in the conjugation of the cells of *Spirogyra*, where the conjugation is undoubtedly part of the process of reproduction. Whether the spore-shaped contents of the cells of *Spirogyra*, which thus appear to be imperfectly formed, ever germinate, has not, to my knowledge, been determined. I should think that at least the smaller portions became abortive.

Lastly, we have to consider the import of the small spherules of the nucleolus. Formerly, I thought that they might be sperm-cells, or impregnating-agents, when studying the germinative process in the Rhizopodous cell that inhabits the protoplasm of *Nitella* (see good drawings of this cell, and a description of them, in 'Annals,' vol. xviii. p. 237, pl. 7. figs. 93–98); but on looking over my sketches of this cell in connexion with what I have lately witnessed in the nucleus of *Difflugia pyriformis*, it seems to me that the "protoplasmic zone," which in this instance becomes mulberry-shaped, and which I then supposed to be developed around (outside) the nucleus, should have been regarded as homologous with the protoplasm in which the spherules of *Difflugia* are developed—that is, as the nuclear protoplasm. Certainly, here, in the Rhizopodous cell of

Nitella, the spherules become the new brood, and, on leaving the nucleus, pass into the effete cell of the parent, where still remain the contents (starch and chlorophyll) originally incepted by it in the internodal cell of its host; after feeding on which (for portions of it may be seen in their bodies), these mono- and diplo-ciliated monads (individuals of the new generation) find their way out through the effete parent-cell, and commence their independent existence.

It is thus, in all probability, that the granuliferous cells in *Difflugia pyriformis* leave the nucleus, multiply rapidly by duplicative division (for their number in the body of the parent far exceeds that of the number of the spherules of the nucleus), feed on the starch laid up for them, and finally, becoming ciliated, leave the effete parent, to obtain their future maintenance and development.

The granulation of the nucleus in *Difflugia*, as before stated, seems to be the same as that which I have described in *Amœba princeps*; but that of generation by the "reproductive cells" in *A. princeps* is altogether a different process. In the former, several centres of new individuals become developed in the protoplasm of the nucleus, while in the latter the nucleus appears to begin to form the "reproductive cells" by dividing at once into two equal portions, and so on. But both processes, in their generative imports respectively, are as yet very imperfectly understood; and at present we know no more of them, as regards impregnation, than we do of that of the conjugating Confervoid Algæ generally, the Diatomaceæ or the Desmidiaceæ.

Observations.—In the starch-granules of *Difflugia pyriformis* I cannot help seeing the refractive "cells" (or, rather, granules), with the small granules and protoplasm, which fill the globular cells of the seed-like body of *Spongilla*, each of which globular cells, in the early stage of this body, is a *bonâ fide Amœba*; and when the new sponge-substance issues from the hilous aperture (that is, when the seed-like body germinates), each of the globular cells (now become effete), with its contents, appears to pass into one of the "ampullaceous sacs" (see a description of this, "Ultimate Structure of Spongilla," 'Annals,' vol. xx. p. 21, 1857), while the refractive cells or granules gradually disappear, and are replaced by the polymorphic cells which chiefly enter into the composition of this "sac." Thus my original view, that these refractive grains are "ovules," is changed, as announced in 1859, in my observations "On the Identity in Structure and Composition of the Seed-like Body of *Spongilla* with the Winter-egg of the Bryozoa, &c." (Annals, vol. iii. p. 331), where I have also stated, for reasons therein mentioned, that these refractive granules appear to pass into the formation of the sponge-cells by the "vito-catalytic" influence of a thin film of protoplasm with which they become surrounded.

Now it seems to me that this thin film may be an expansion of one of the "small granules" of the globular cell of the seed-like body, and that these granules might have originated from a granulation of the nucleus of the globular cells respectively; for certainly no trace remains of the nucleus, which as certainly existed when the globular cell was in an active state. It must be understood here that the "granule" is viewed as a nucleus, or centre of vitality, capable of developing a cell around itself in the manner of the germ of the "cell" generally, which membrane in this instance would be plastic and polymorphic, and therefore become expansible into the thin film mentioned.

In this way we should have a direct analogy between the development of the amœbous cell, which, in its plurality, makes up a great part of the ampullaceous sac of *Spongilla,* and that of the new granuliferous cells of the colourless *Difflugia pyriformis,* assuming that the granuliferous cells are the new brood, and that in both instances, viz. in the amœbous cells of the ampullaceous sac and the granuliferous cells of *Difflugia,* respectively, the germs are derived from a granulation of the nucleus.

Further, should the conjugation of the tests of *Difflugia pyriformis* be hereafter shown to be connected with the impregnating process, then it may be fairly assumed that a similar conjugation takes place between two reproductive cells of *Spongilla,* which may lead to a granulation of the nucleus there also, and this, again, to the production of the number of amœbous cells which make up the chief bulk of the seed-like body at the commencement, and which, after the capsule has been secreted round them, ultimately enlarge and pass into the globular cells which fill the cavity of the seed-like body, and which, lastly, in their turn, on the issue of the sponge-substance, follow the development above mentioned.

If, then, the refractive "cells" or granules of the globular cells of the seed-like body in *Spongilla* be the same as the refractive amylaceous granules of *Difflugia pyriformis,* then in those *Amœbæ* in which I have described them as "ovules," and wherever they occur in the Rhizopoda, the same view must be taken of them, viz. that they pertain to the nature of, if they be not fully developed starch-granules.

I long since pointed out that *Spongilla* abounds with starch in all forms. Auerbach has demonstrated it in *Amœba bilimbosa,* and I have also shown that it exists in the chambers of the Foraminifera (viz. in *Operculina Arabica*), and now in *Difflugia pyriformis.*

Again, as in *Euglena* (where I also formerly thought similar refractive cells might be "ovules"), it seems to me now that they might also be considered as analogous to the refractive granules of *Spongilla*, &c. (that is, of an amylaceous composition), which, with the chlorophyll-vesicles of *Euglena*, would then constitute just as much a part of this and all similar organisms as the chlorophyll-cells and starch-granules have been shown to do of the body of *Difflugia pyriformis*.

Lastly, when we extend this analogy to the *Euglenæ*, &c., it passes us on to *Spirogyra*, *Œdogonium*, and the composition of the contents of the cells of all the Confervoid Algæ, where we find all these refractive granules are actually composed of genuine starch, as much as in the common vegetable cell.

Résumé.—This article is to show—

1. That chlorophyll-cells exist in the body of *Difflugia pyriformis* as part of its organization.
2. That starch-granules form part of its products.
3. That the tests conjugate.
4. That, apparently after this conjugation, when the body of the *Difflugia* is densely charged with chlorophyll-cells and starch-granules, the nucleus becomes charged with spherular, refractive, homogeneous bodies, which appear to be developed in the protoplasm that lines (?) the nucleus.
5. That the spherules pass from the nucleus into the body of the animal, and there, becoming granuliferous, so increase by duplicative division as to form the chief bulk of the whole mass, while the chlorophyll-cells have *entirely* disappeared, and the starch-granules have become more or less diminished in number.

It now remains to be shown whether the granuliferous cells become polymorphic and ciliated, like the spherules in the Rhizopodous cell of *Nitella*, and, finally, whether they pass into young *Difflugiæ*,—for which purpose I have collected a great many of the tests, both green and colourless, and have placed them aside for observation.

The first question is thus answered:—

Since the above was written, the bottom of a watch-glass, in which four of the colourless specimens were placed with a little water four days ago, has become covered with granuliferous cells of the same size and appearance as those peculiar to the colourless specimens, but with the following differences, viz. that they are all provided with a cilium (perhaps two); most are fixed to the watch-glass, and retain their globular form; others are swimming about by means of their cilium; many of the fixed globular forms are altering their shape by becoming polymorphic; and some have lost their cilium, and have become altogether reptant and amœbous. Their sizes average between $\frac{1}{6000}$th and $\frac{1}{1200}$th part of an inch in diameter, the former being that of the globular, and the latter that of the plane or amœbous forms. This has been confirmed by a repetition of this experiment.

Now, as the granuliferous cells on the watch-glass are so much like those in the interior of the colourless specimens of *Difflugia pyriformis* (which granuliferous cells have heretofore been inferred to have come from the spherules of the nucleus in the coloured or green state of the animal), and there is no other source in the watch-glass, apparently, from which they could have been derived, while the four *Difflugiæ* are still alive (for this is a necessary adjunct, since we are now among a class of beings where the death of one frequently affords nutriment for the almost instantaneous evolution of another species), and a gelatinous mass is projecting from their apertures respectively, which may also be inferred to be the protoplasm charged with the granuliferous cells, there can be no reasonable doubt that the granuliferous cells of the watch-glass came from these *Difflugiæ*. And when we find that a cilium is added to them, that they are polymorphic, and that some have lost this cilium and have assumed an amœbous state, which strictly accords with what has been seen in the generative development of the rhizopodous cell of *Nitella*, there can be just as little doubt that these *Amœbæ* are the young brood of *Difflugia pyriformis*. Thus the cycle of generative development in *Difflugia pyriformis*, by "granulation of the nucleus," is so far completed. It is probably the same in *Amœba princeps*.

The next step will be to follow the development of the young *Amœbæ* into the adult testaceous *Difflugia*. But this will be much more difficult, since it may not take place for many months, during which time these little *Amœbæ* may become *pro tempore* inhabitants, and probably subsequent destroyers, of vegetable cells into which they have penetrated for nutriment. Myriads, of course, as in every other case, are themselves destroyed by the contingencies which intervene between infancy and adult age.

While studying *Difflugia pyriformis*, it has been my good fortune to meet with two specimens of another Rhizopod containing chlorophyll-cells and refractive amylaceous granules as normal parts of the animal; but this novelty, if such it may now be termed, does not rest here; for this Rhizopod, which I at first thought to be a loricated *Actinophrys*, proves, on further examination, to be so nearly allied to *Acanthometra* among the Echinocystidia, that I cannot help viewing it as a freshwater species of this order, and shall for the present describe it as

Acanthocystis turfacea, n. sp. et gen.?

Globular, subround, of a green colour, loricated, spiniferous, and tentaculiferous. Lorica flexible, covered with minute, fusiform, slightly curved spicules, which give the outline a fibrous wavy appearance. Spines straight, hollow, of uniform breadth in the shaft, bifid or forked at the distal, and discoid at the proximal extremity, which rests upon the lorica; very numerous, apparently rigid, radiating or turned across each other and moveable as the spines in *Echinus*. Tentacula three times the length of the spines, colourless, delicate, rough or granular, and retractile. Interior of the body lined with granular protoplasm, chlorophyll-cells, and refractive colourless amylaceous granules. Nucleus peripheral? Contracting vesicle also peripheral, and in plurality, if certain temporary and conical projections of the lorica indicate this.

Size. Lorica $\frac{1}{411}$th of an inch in diameter; fusiform spicules $\frac{1}{8000}$th long; spines $\frac{1}{740}$th long, and disk of same $\frac{1}{14800}$th, or twice the width of the shaft; tentacles $\frac{1}{246}$th of an inch long.

Hab. Heath-bog water. Locomotive; progressing by means of the spines, which are ambulacral, and by the tentacles(?). Kind of nutriment and mode of incepting the same undetermined; probably in very minute portions, or by suction, as with *Acineta*.

Loc. South coast of Devon.

Observations.—I have found several specimens of this Rhizopod, one of which was one-third larger than the measurements above given; some are colourless. It at first looks like a spiniferous *Actinophrys*; and not unlike the figure of *A. viridis*, Ehr.

Dilute sulphuric acid, followed by iodine, gives a deep claret-colour to the refractive granules, and a dark brown colour to the chlorophyll-cells, which are thus seen to contain a granular protoplasm. Strong sulphuric acid colours the substance of the tubes apparently black, but, after deepening, extracts the colour of the refractive granules entirely. On the addition of more iodine (*i. e.* iodine in solution of iodide of potassium), the dark colour of the interior of the spines disappears from both ends towards the centre, thus showing that the spines are hollow. Neither strong sulphuric nor nitric acid dissolves the spines nor the "fusiform spicules" on the lorica; while their rigid nature, in addition, inclines to the view that they are both siliceous. During life, the free or forked extremity of some of the spines is closed and pointed. The tentacles disappear, and some of the spines (which are much longer than the general average in certain specimens) become detached under the effect of the acid, when they are easily examined. I have not been able to see any tentacles projected through the spines, as in *Acanthometra*, nor do the spines extend further inwards than the lorica. The whole organism very much resembles in its capsular elements those of the seed-like body of *Spongilla Meyeni*. I saw no crude food in the interior; but the nature of the nutriment, as well as other points in the history of this Rhizopod, may be elucidated by further examination.

XXV.—*Notice of a* Drassus *and* Linyphia *new to Science, and a* Neriene *hitherto unrecorded as British*. By John Blackwall, F.L.S.

Tribe Octonoculina.

Family Drassidæ.

Genus Drassus, Walck.

Drassus gracilipes.

Length of an immature male $\frac{3}{16}$ths of an inch; length of the cephalothorax $\frac{1}{14}$; breadth $\frac{1}{16}$; breadth of the abdomen $\frac{1}{16}$; length of a posterior leg $\frac{1}{3}$; length of a leg of the third pair $\frac{1}{5}$.

The cephalothorax is convex, compressed before, rounded in front and on the sides, with slight furrows on the latter converging towards a narrow indentation in the medial line; it is soot-coloured, sparingly clothed with white hairs, and has a narrow fringe of hairs of the same hue on the lateral margins. The falces are conical and vertical; the maxillæ are convex at the base, and somewhat inclined towards the lip, which is nearly quadrate, and slightly hollowed at the apex. These parts are of a brown hue, the inner side of the maxillæ and the extremity of the lip and falces being much the palest. The sternum is heart-shaped, with small eminences on the sides opposite to the legs, and is soot-coloured, but rather browner than the cephalothorax. The eyes are disposed on the anterior part of the cephalothorax in two transverse, slightly curved, nearly parallel rows, the anterior row being rather the more curved; the lateral eyes are the largest, and the intermediate ones of the anterior row are the smallest and darkest of the eight. The legs are long, slender, and provided with hairs and spines, two parallel rows of long sessile spines occurring on the inferior surface of the tibiæ and metatarsi of the first and second pairs; the fourth pair is the longest, then the first, and the third pair is the shortest; each tarsus is terminated by two small curved claws, below which there is a minute scopula; the anterior legs have a black hue, with the exception of the base of the genual joint,